

1. A method for the extraction of true values of emission anisotropy ($\langle r \rangle_{\text{corr}}$) from fluorescence intensities obtained for a sample under an applied hydrostatic pressure (p), comprising the steps of measuring polarized fluorescence intensities and then determining excitation and emission correction factors.

3. The method of claim 1 wherein said excitation correction factor X and said emission correction factor Y are determined for a given pressure (p) from said fluorescence intensities substantially according to the equations:

$$X(p) = \frac{G \cdot i_{HV} - i_{HH}}{G \cdot i_{HV} - i_{HH} + E \cdot (G \cdot i_{VV} - i_{VH})}$$

$$Y(p) = \frac{E \cdot i_{VH} - i_{HH}}{E \cdot i_{VH} - i_{HH} + G \cdot (E \cdot i_{VV} - i_{HV})}$$

4. The method of claim 3 wherein the *E*-factor corrects for any inequality in the intensities of the vertical and horizontal polarized excitation light, the *G*-factor corrects for unequal sensitivity of the detection system to the vertical and horizontal polarized

4. The method of claim 3 wherein the *E*-factor corrects for any inequality in the intensities of the vertical and horizontal polarized excitation light, the *G*-factor corrects for unequal sensitivity of the detection system to the vertical and horizontal polarized

emission light, and said E and G factors are determined at atmospheric pressure according to the equations:

$$G = \frac{i_{HH_0}}{i_{HV_0}} \quad \text{and} \quad E = \frac{i_{HH_0}}{i_{VH_0}}$$

where said i_{VH0} , i_{HH0} , and i_{HV0} are polarized fluorescence intensities obtained at atmospheric pressure.

- 5 5. The method of claim 3, further comprising the use of said excitation and emission correction factors to detect abnormalities in an optical window.

6. The method of claim 4 wherein said true values of emission anisotropy ($\langle r \rangle_{\text{corr}}$) are obtained from the equations:

$$\langle r \rangle_{corr} = \frac{R - 1}{R + 2 - 3 \cdot (X + Y - X \cdot Y + R \cdot Y - R \cdot X \cdot Y)}; \quad R = G \cdot \frac{i_{VV}}{i_{VH}}$$

7. The method of claim 4, further comprising determining corrected total
10 intensities (S_{corr}) in accordance with the following formula:

$$S_{corr} = G \cdot \frac{1-3 \cdot (Y-X \cdot Y)}{1-X-2 \cdot (Y-X \cdot Y)} \cdot i_{vv} + \frac{2-3 \cdot (X+Y-X \cdot Y)}{1-X-2 \cdot (Y-X \cdot Y)} \cdot i_{vh}$$

8. A method for the extraction of corrected values of total intensities (S_{corr}) from polarized fluorescence intensities obtained for a sample under an applied hydrostatic pressure (p), comprising the steps of measuring polarized fluorescence intensities and then determining excitation and emission correction factors.

10. The method of claim 8 wherein said excitation correction factor X and said
5 emission correction factor Y are determined for a given pressure (p) from said
fluorescence intensities substantially according to the equations:

and:

wherein i_{VV} , i_{VH} , i_{HH} , and i_{HV} represent the measured and distorted polarized intensities for the sample of interest, and E and G , are both sample and pressure independent instrument factors characteristic for the chosen excitation and emission wavelength conditions.

$$G = \frac{i_{HH_0}}{i_{HV_0}} \quad \text{and} \quad E = \frac{i_{HH_0}}{i_{VH_0}}$$

where said i_{VH0} , i_{HH0} , and i_{HV0} are polarized fluorescence intensities obtained at atmospheric

pressure.

12. The method of claim 10, further comprising the use of said excitation and emission correction factors to detect abnormalities in an optical window.

13. The method of claim 8 wherein said total intensities (S_{corr}) are obtained
5 substantially from the equation:

$$S_{corr} = G \cdot \frac{1 - 3 \cdot (Y - X \cdot Y)}{1 - X - 2 \cdot (Y - X \cdot Y)} \cdot i_{VV} + \frac{2 - 3 \cdot (X + Y - X \cdot Y)}{1 - X - 2 \cdot (Y - X \cdot Y)} \cdot i_{VH}$$

14. A process for measuring and removing scrambling effects, induced by an applied hydrostatic pressure (p), from fluorescence intensities while avoiding the need for a separate pressurized calibration experiment, comprising the acts of measuring polarized fluorescence intensities and then determining excitation and emission correction factors
10 simultaneously.

15. A process as recited in claim 14, wherein the act of determining excitation and emission correction factors simultaneously comprises the determination of excitation ($X(p)$) and emission ($Y(p)$) components the respective values of which are dependent on hydrostatic pressure.

16. A process as recited in claim 14, wherein $X(p)$ is given by:

$$X(p) = \frac{G \cdot i_{HV} - i_{HH}}{G \cdot i_{HV} - i_{HH} + E \cdot (G \cdot i_{VV} - i_{VH})}$$

wherein E and G , are both sample and pressure independent instrument factors characteristic for the chosen excitation and emission wavelength conditions, and HV, HH, VV and VH are polarized fluorescence intensities.

17. A process as recited in claim 14, wherein $Y(p)$ is given by:

$$Y(p) = \frac{E \cdot i_{VH} - i_{HH}}{E \cdot i_{VH} - i_{HH} + G \cdot (E \cdot i_{VV} - i_{HV})}$$

wherein E and G , are both sample and pressure independent instrument factors characteristic for the chosen excitation and emission wavelength conditions, and VH, HH, VV and HV are polarized fluorescence intensities.

18. A process as recited in claim 1, further comprising determining a steady
5 state fluorescence emission anisotropy value ($\langle r \rangle_{\text{corr}}$).

19. A method for obtaining the true difference in polarized fluorescence intensities (D) from fluorescence intensities obtained for a sample under an applied hydrostatic pressure (p), comprising the steps of measuring polarized fluorescence intensities and then determining excitation and emission correction factors.

- 10 20. The method of claim 19 wherein said true difference in polarized
fluorescence intensities (D) are obtained from said fluorescence intensities without
performing a separate pressurized calibration experiment.

21. The method of claim 19 wherein said excitation correction factor X and said emission correction factor Y are determined for a given pressure (p) from said
15 fluorescence intensities substantially according to the equations:

$$X(p) = \frac{G \cdot i_{HV} - i_{HH}}{G \cdot i_{HV} - i_{HH} + E \cdot (G \cdot i_{VY} - i_{VH})}$$

and:

$$Y(p) = \frac{E \cdot i_{VH} - i_{HH}}{E \cdot i_{VH} - i_{HH} + G \cdot (E \cdot i_{VV} - i_{HV})}$$

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wherein i_{VV} , i_{VH} , i_{HH} , and i_{HV} represent the measured and distorted polarized intensities for the sample of interest, and E and G , are both sample and pressure independent instrument factors characteristic for the chosen excitation and emission wavelength conditions.

22. The method of claim 19 wherein the E -factor corrects for any inequality in the intensities of the vertical and horizontal polarized excitation light, the G -factor corrects for unequal sensitivity of the detection system to the vertical and horizontal polarized emission light, and said E and G factors are determined at atmospheric pressure according to the equations:

$$G = \frac{i_{HH_0}}{i_{HV_0}} \quad \text{and} \quad E = \frac{i_{HH_0}}{i_{VH_0}}$$

- where said i_{VH_0} , i_{HH_0} , and i_{HV_0} are polarized fluorescence intensities obtained at atmospheric pressure.

23. The method of claim 19 wherein said difference in polarized fluorescence intensities (D) are obtained substantially from the equation:

24. A method for the correction of time dependent polarized fluorescence intensities obtained for a sample under an applied hydrostatic pressure (p), comprising the steps of:
- a) collecting four non-truncated polarized (i_{VV} , i_{VH} , i_{HH} , i_{HV}) decay profiles;
 - b) integrating said decay profiles;
 - c) calculating emission and excitation correction factors X and Y , respectively, from integrals of said profiles; and
 - d) using said emission and excitation factors, together with said i_{VV} and i_{VH} decay profiles, to perform a sum-difference analysis to obtain profiles for total corrected intensity (S_{corr}) and difference in polarized fluorescence intensity (D_{corr}).

25. The method of claim 24 wherein said correction is performed without performing a separate pressurized calibration experiment.

26. The method of claim 24 wherein said excitation correction factor X and said emission correction factor Y are determined for a given pressure (p) from said
5 fluorescence intensities substantially according to the equations:

$$X(p) = \frac{G \cdot i_{HV} - i_{HH}}{G \cdot i_{HV} - i_{HH} + E \cdot (G \cdot i_{VV} - i_{VH})}$$

and:

$$Y(p) = \frac{E \cdot i_{VH} - i_{HH}}{E \cdot i_{VH} - i_{HH} + G \cdot (E \cdot i_{VV} - i_{HV})}$$

wherein i_{VV} , i_{VH} , i_{HH} , and i_{HV} represent the measured and distorted polarized intensities for the sample of interest, and E and G , are both sample and pressure independent instrument factors characteristic for the chosen excitation and emission wavelength conditions.

10 27. The method of claim 26 wherein the E -factor corrects for any inequality in the intensities of the vertical and horizontal polarized excitation light, the G -factor corrects for unequal sensitivity of the detection system to the vertical and horizontal polarized emission light, and said E and G factors are determined at atmospheric pressure according to the equations:

$$G = \frac{i_{HH_0}}{i_{HV_0}} \quad \text{and} \quad E = \frac{i_{HH_0}}{i_{VH_0}}$$

15 where said i_{VH_0} , i_{HH_0} , and i_{HV_0} are polarized fluorescence intensities obtained at atmospheric pressure.

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28. The method of claim 27 wherein said difference in polarized fluorescence intensities (D) are obtained substantially from the equation:

$$D_{corr} = G \cdot \frac{1}{1-X-2 \cdot (Y-X \cdot Y)} \cdot i_{VV} - \frac{1}{1-X-2 \cdot (Y-X \cdot Y)} \cdot i_{VH}$$

29. A computer readable storage medium comprising computer executable code for instructing a computer-controlled instrument to perform the acts of measuring polarized fluorescence intensities and then determining excitation and emission correction factors.

30. The computer readable storage medium of claim 29 wherein said excitation correction factor X and said emission correction factor Y are determined for a given pressure (p) from said fluorescence intensities substantially according to the equations:

$$X(p) = \frac{G \cdot i_{HV} - i_{HH}}{G \cdot i_{HV} - i_{HH} + E \cdot (G \cdot i_{VV} - i_{VH})}$$

and:

$$Y(p) = \frac{E \cdot i_{VH} - i_{HH}}{E \cdot i_{VH} - i_{HH} + G \cdot (E \cdot i_{VV} - i_{HV})}$$

wherein i_{VV} , i_{VH} , i_{HH} , and i_{HV} represent the measured and distorted polarized intensities for the sample of interest, and E and G , are both sample and pressure independent instrument factors characteristic for the chosen excitation and emission wavelength conditions.

31. The computer readable storage medium of claim 30 wherein the E -factor corrects for unequal sensitivity of the detection system to the vertical and horizontal polarized excitation light, the G -factor corrects for any inequality in the intensities of the

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vertical and horizontal polarized emission light, and said E and G factors are determined at atmospheric pressure according to the equations:

$$G = \frac{i_{HH_0}}{i_{HV_0}} \quad \text{and} \quad E = \frac{i_{HH_0}}{i_{VH_0}}$$

where said i_{VH_0} , i_{HH_0} , and i_{HV_0} are polarized fluorescence intensities obtained at atmospheric pressure.

- 5 32. The computer readable storage medium of claim 31, further comprising the use of said excitation and emission correction factors to detect abnormalities in an optical window.

33. The computer readable storage medium of claim 31 wherein said true values of emission anisotropy ($\langle r \rangle_{\text{corr}}$) are obtained from the equations:

$$\langle r \rangle_{\text{corr}} = \frac{R - 1}{R + 2 - 3 \cdot (X + Y - X \cdot Y + R \cdot Y - R \cdot X \cdot Y)} ; \quad R = G \cdot \frac{i_{VV}}{i_{VH}}$$

- 10 34. The computer readable storage medium of claim 33 wherein said true values of emission anisotropy are obtained from said fluorescence intensities without performing a separate pressurized calibration experiment.

35. The computer readable storage medium of claim 34, further comprising determining corrected total intensities (S_{corr}) in accordance with the following formula:

$$S_{\text{corr}} = G \cdot \frac{1 - 3 \cdot (Y - X \cdot Y)}{1 - X - 2 \cdot (Y - X \cdot Y)} \cdot i_{VV} + \frac{2 - 3 \cdot (X + Y - X \cdot Y)}{1 - X - 2 \cdot (Y - X \cdot Y)} \cdot i_{VH}$$

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36. A computer-controlled instrument for measuring and removing scrambling effects, induced by an applied hydrostatic pressure (p), from fluorescence intensities while avoiding the need for a separate calibration experiment, comprising a computer/ processor, a fluorescence spectrometer, and a computer readable storage medium comprising
5 computer executable code for instructing the instrument to perform the acts of measuring polarized fluorescence intensities and then determining excitation and emission correction factors.

37. The computer controlled instrument of claim 1 wherein said excitation correction factor X and said emission correction factor Y are determined for a given
10 pressure (p) from said fluorescence intensities substantially according to the equations:

$$X(p) = \frac{G \cdot i_{HV} - i_{HH}}{G \cdot i_{HV} - i_{HH} + E \cdot (G \cdot i_{VV} - i_{VH})}$$

and:

$$Y(p) = \frac{E \cdot i_{VH} - i_{HH}}{E \cdot i_{VH} - i_{HH} + G \cdot (E \cdot i_{VV} - i_{HV})}$$

wherein i_{VV} , i_{VH} , i_{HH} , and i_{HV} represent the measured and distorted polarized intensities for the sample of interest, and E and G , are both sample and pressure independent instrument factors characteristic for the chosen excitation and emission wavelength conditions.

15 38. The computer readable storage medium of claim 36 wherein the E -factor corrects for any inequality in the intensities of the vertical and horizontal polarized excitation light, the G -factor corrects for unequal sensitivity of the detection system to the vertical and horizontal horizontal polarized emission light, and said E and G factors are determined at atmospheric pressure according to the equations:

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